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Life Cycle of *Amphimerus elongatus* (Trematoda: Opisthorchiidae)

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ABSTRACT: Life cycle of *Amphimerus elongatus* and seasonal occurrence of cercariae and metacercariae were studied in Half Moon Lake, Eau Claire, Wisconsin from September 1982 to November 1984. Oculate pleurolophocercous cercariae shed from naturally infected *Amnicola limosa* were positively phototactic. Cercariae possessed 14 penetration glands with ducts terminating anteriorly in a 3:4:4:3 pattern, similar to that described for *Clonorchis sinensis*. Peak of cercarial production occurred in midsummer. Nearly all centrarchid fishes collected from Half Moon Lake were infected with metacercariae of *A. elongatus*. *Lepomis macrochirus, L. gibbosus,* and *Micropterus salmoides* exposed to cercariae in laboratory experiments proved to be the most susceptible second intermediate hosts. Prevalence in experimentally infected juvenile bluegills was 100% with an average intensity of 10.5 metacercariae. Two cyprinids, *Pimephales promelas* and *Rhinichthys cataractae,* were less suitable as hosts for metacercariae, and no other fishes exposed to cercariae in the laboratory could be infected. Metacercariae were infective 1 month postexposure. Adult *A. elongatus* were recovered from liver and pancreas of chickens and house sparrows fed metacercariae from naturally and experimentally infected fishes.

KEY WORDS: *Amphimerus elongatus,* Opisthorchiidae, *Amnicola limosa,* Hydrobiidae, life cycle, seasonal dynamics, natural infections, experimental infections, cercaria, metacercaria, *Lepomis macrochirus, Micropterus salmoides,* fish, waterfowl.

Amphimerus elongatus Gower, 1938, was originally described from specimens obtained from liver and pancreas of wild, captive, and experimentally infected ducks and swans at the W. K. Kellogg Bird Sanctuary, Michigan (Gower, 1938a). Gower infected mallards, *Anas platyrhynchos,* by feeding them various species of fish but was not successful in determining specifically which fish served as second intermediate hosts. Subsequently, Gower (1938b) reported briefly the seasonal prevalence of *A. elongatus* in ducks from the wildlife sanctuary. He found prevalences of 11.5%, 2%, and 30% in summer, fall, and winter, respectively.

Wallace (1939) described the metacercaria of *A. elongatus* from naturally infected minnows, *Notropis deliciosus stramineus,* in Minnesota. Adult worms which he obtained from experimentally infected chicks and ducks were morphologically similar to specimens described by Gower. Cameron (1944) incidentally reported the occurrence of metacercariae in suckers in Canada. Boyd and Fry (1971) reported adult *A. elongatus* from the common loon, *Gavia immer,* and the belted kingfisher, *Megaceryle alcyon.* Pence and Childs (1972) described the tissue damage to the liver of a double-crested cormorant, *Phalacrocorax auritus,* caused by a massive infection of *A. elongatus.* Nothing has been published on the mollusc first intermediate host or cercaria of *A. elongatus* except for a single statement in an abstract by Wallace (1940): "pleurolophocercous distome cercariae with 14 penetration glands from *Amnicola limosa portata* develop in fish into metacercariae identical with those of *A. elongatus.*"

Weil et al. (1986) reported the pathophysiology of *A. elongatus* in experimentally infected chicks. Their study was made possible by the discovery of *A. elongatus* metacercariae in bluegills, *Lepomis macrochirus,* in Half Moon Lake, Eau Claire, Wisconsin. Because only limited life history information has been reported for this parasite, a study of the life cycle of *A. elongatus* was initiated at this study site and is reported in this paper.

Materials and Methods

Natural infections

Hydrobiid snails identified as *Amnicola limosa* (Say) were collected with dipnets and by hand from Half Moon Lake, Eau Claire, Wisconsin, and maintained in 38-liter aerated aquaria. Voucher specimens of *A. limosa* were relaxed with menthol crystals, preserved in 70% ethanol, and deposited in the Malacology Collection, Florida State Museum, Gainesville (UF 40463). Snails were isolated individually in 37-mm diameter Stender dishes and induced to shed cercariae by exposure to bright sunlight or artificial light.

Bluegills, *Lepomis macrochirus* Rafinesque, large-

mouth bass, *Micropterus salmoides* (Lacépède), white crappie, *Pomoxis annularis* Rafinesque, black bullheads, *Ictalurus melas* (Rafinesque), and northern pike, *Esox lucius* Linnaeus, were collected from Half Moon Lake with dipnets, castnets, seines, or by ice fishing in winter. Fins and musculature were examined for metacercariae. Survival of metacercariae in dead fish maintained at 4°C was determined by microscopic observation of movement of the whole worm within the cyst and by observation of flame cell motility.

A limited search for natural definitive hosts of *A. elongatus* was made by examining 9 hunter-killed ducks from north central Wisconsin; however, no birds from Half Moon Lake, located in the city park, were collected. Observations on birds foraging in the lake were made in all seasons of the year.

Experimental infections

Fish collected from locations that were determined to be free from *A. elongatus* by examination of at least 10 specimens of each fish species, were exposed in groups of 20 to more than 1,000 cercariae in 4-liter aquaria for 24 hr. Bluegill, *Lepomis macrochirus*, pumpkinseed, *L. gibbosus* (Linnaeus), black bullhead, *Ictalurus melas*, longnose dace, *Rhinichthys cataractae* (Valenciennes), and emerald shiner, *Notropis atherinoides* Rafinesque, were collected in Fall Creek, town of Fall Creek, Wisconsin. Fantail darter, *Etheostoma flabellare* Rafinesque, Johnny darter, *E. nigrum* Rafinesque, and mudminnow, *Umbra limi* (Kirtland), were collected in O'Neil Creek, 5 km N Chippewa Falls, Wisconsin. Fathead minnow, *Pimephales promelas* Rafinesque, and largemouth bass, *Micropterus salmoides*, were collected in Frontage Road Pond, 3 km S of Eau Claire. Brook stickleback, *Culaea inconstans* (Kirtland), and creek chub, *Semotilis atromaculatus* (Mitchell), were collected in Little Niagara Creek, Eau Claire. After exposure, fish were maintained in aerated 38-liter aquaria and fed flake fish food. Fish were examined for metacercariae at various intervals from day 1 through day 68 postexposure (PE).

Metacercariae from both naturally infected and experimentally infected fishes were fed to young chicks, adult chickens, house sparrows, mice, rats, and cats in the laboratory.

Parasite morphology

Cercariae were studied alive with the aid of bright field, phase contrast, and differential interference contrast optics. Neutral red and Nile blue were employed as vital stains. Twenty cercariae were fixed in steaming 10% formalin, mounted under a floating coverslip, and measured using an ocular micrometer.

Metacercarial cysts from naturally infected fish were measured alive under floating coverslip ($N = 20$). Over 100 metacercariae from experimentally infected fish were measured at various intervals postexposure. Metacercariae were excysted artificially at 38°C in a solution of trypsin and bile salts (Irwin, 1983). Excysted metacercariae and adults were killed with steaming (160°C) AFA, stained with Semichon's carmine, and mounted in Permount. Unless otherwise indicated, measurements are expressed in micrometers, the range followed by mean in parentheses. Drawings were made with a microprojector.

Results

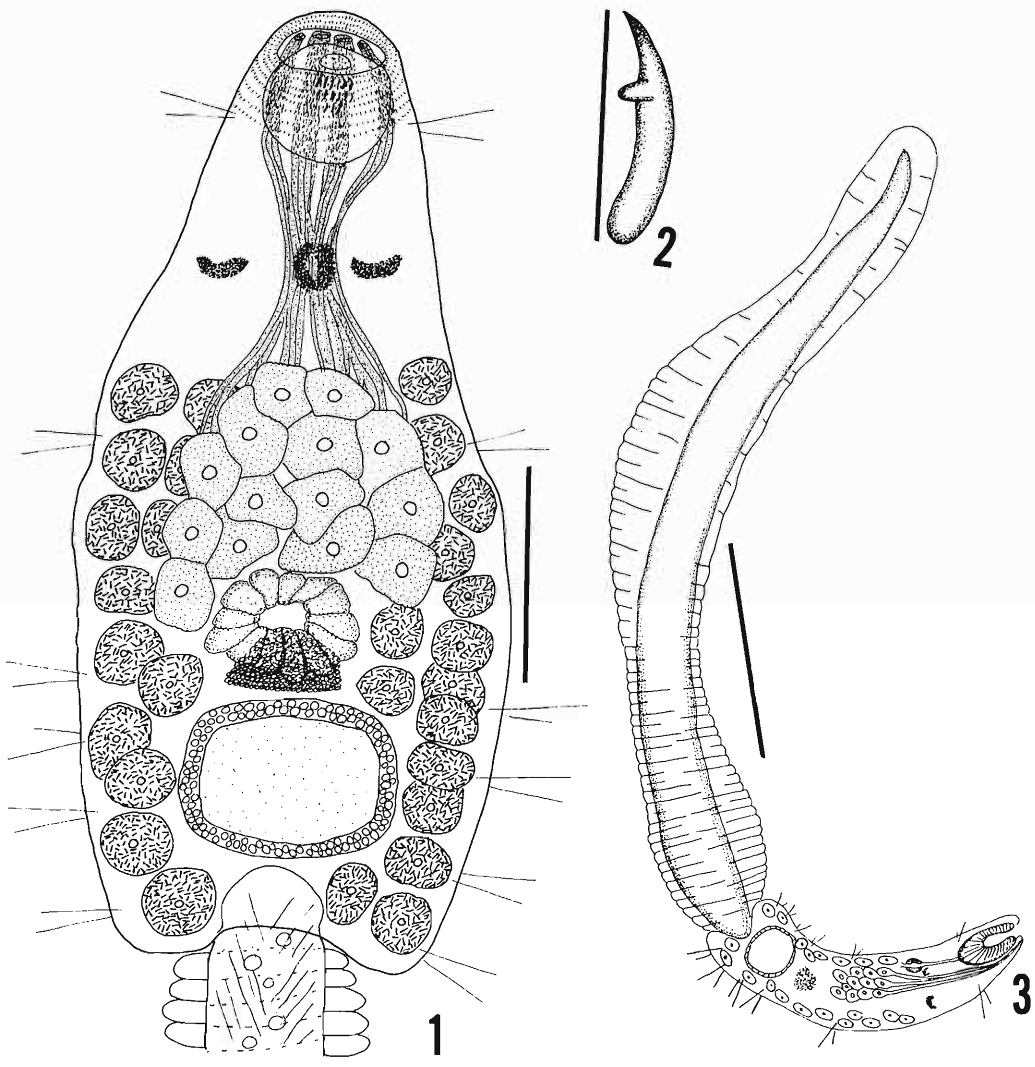
Cercaria (Figs. 1–3)

Body spinose anteriorly, oculate, pleurolophocercous, 193–243 (215) long by 53–75 (66) wide; with paired sensillae on lateral margins, 1 pair near oral sucker, 1 pair near midbody, 4 pairs in hindbody (Fig. 1). Oral sucker, protrusible from tegumental hood 28–38 (35) long by 25–33 (29) wide bearing 3 rows of small teeth (Fig. 2) on oral sucker, 8 per row anteriormost to 3 per row posteriormost. Acetabulum rudimentary, in tegumental fold at midbody. Genital anlage overlapping acetabulum. Pharynx situated medially between eyespots; esophagus and ceca undifferentiated. Fourteen penetration glands, staining weakly, clustered medially between ventral sucker and pharynx; ducts terminating anterior to oral sucker forming 3:4:4:3 pattern. Thirteen to 17 pairs of granular cystogenous glands staining intensely with vital stains, occupying dorsolateral margins of mid- and hindbody. Wall of translucent excretory vesicle epithelial with 2 rows of conspicuous spherical nuclei; ovoid, bounded anteriorly by genital anlage and posteriorly by tail insertion. Excretory ducts and flame cell pattern obscured by coarse granules of cystogenous gland cells. Tail elongate 400–475 (435) long by 28–38 (33) wide, entirely covered by dorsoventral finfold with numerous transverse striations (Fig. 3). Finfold widest in midregion of dorsal margin.

Cercariae emerged from the snail, *Amnicola limosa* (Hydrobiidae), in the laboratory under the stimulus of bright light, with emergence commencing within 15 min of stimulation. Cercariae were positively phototactic. They remained motionless, suspended with body oriented downward, and slowly settled in the water column. They rose in the water column by occasional bursts of swimming activity, simultaneously moving toward the light. Cercariae were able to swim actively for approximately 24 hr. Moribund cercariae lost their tails and crawled weakly on the bottom prior to death.

Metacercaria

Measurements of metacercariae encysted in naturally infected bluegills averaged 185 by 170 with a parasite cyst wall 12 thick. Dimensions of cysts and morphology of excysted metacercariae were similar to those described by Wallace (1939).



Figures 1–3. Cercaria of *Amphimerus elongatus* from *Amnicola limosa,* Half Moon Lake, Eau Claire, Wisconsin. 1. Body of cercaria, ventral view. Scale = 50 μm. 2. Rasping tooth from oral hood. Scale = 4 μm. 3. Resting posture of cercaria, lateral view. Scale = 110 μm.

Adult

No naturally infected definitive hosts were found during this study. Confirmation of the identity of *Amphimerus elongatus* was based upon adult specimens obtained from experimentally infected hosts.

Natural infections in snails

Amnicola limosa from Half Moon Lake initially shed *Amphimerus elongatus* cercariae in fall 1982. *Amnicola limosa* displayed a seasonal population cycle in Half Moon Lake similar to that reported for the species in Rhode Island (Kesler, 1980). Most snails lived for 1 year. Snails present after ice melted in April were hatched in July of the previous summer. As lake temperatures rose (18°C in mid-May), egg laying commenced and continued for 2 mo. Snails ceased laying eggs and began dying in July when temperatures reached 26°C. Few adult snails survived into the fall. Juvenile snails grew rapidly

Table 1. Prevalence of *Amnicola limosa* from Half Moon Lake, Eau Claire, Wisconsin shedding *Amphimerus elongatus* cercariae.

Date	Number examined	Number shedding	Prevalence (%)
27 Sep 1982	100	3	3
24 Apr 1983	200	0	0
25 May 1983	72	4	6
6 Jun 1983	200	15	8
6 Jul 1983	341	60	18
9 Jul 1983	50	10	20
21 Jul 1983	144	39	27
30 Jul 1983	54	26	48
12 Aug 1983	72	10	14
4 Sep 1983	72	1	1
2 Oct 1983	72	0	0
7 Apr 1984	10	0	0
29 May 1984	164	0	0
4 Jun 1984	119	5	4
13 Jun 1984	134	6	4
19 Jun 1984	137	8	6
26 Jun 1984	139	16	12
6 Jul 1984	200	13	7
19 Jul 1984	63	5	8
26 Jul 1984	147	12	8
15 Aug 1984	108	8	7
15 Sep 1984	72	1	1
8 Oct 1984	144	2	1

and by late fall some attained adult size. Final growth of this generation resumed in spring and egg laying by this generation was initiated in May.

The seasonal dynamics of cercarial production was closely related to the 1-yr life span of *Amnicola limosa.* Few snails shed *Amphimerus elongatus* cercariae in April, May, and June of both 1983 and 1984, but by late July prevalence of snails shedding *A. elongatus* peaked (Table 1). The remaining adult snails continued to shed cercariae in August and into the fall; however, so few adult snails were still alive at this time that cercarial production was greatly reduced. By fall the size of the smallest specimens of remaining adults overlapped the size of the largest snails belonging to the new generation. Therefore, prevalence of snails shedding cercariae could not be determined separately for the 2 generations. It could be ascertained qualitatively, however, that the only snails that were shedding cercariae were unequivocally old adults. These were the largest specimens and had heavily eroded and encrusted shells. Similarly, none of the snails belonging to the most recent generation shed *A. elongatus.* The pattern of cercarial shedding by *Amnicola limosa,* i.e., emergence beginning in late May and peaking in July, was similar in both years of this study, although prevalence of infected snails was much higher in 1983 than in 1984.

Table 2. Distribution of *Amphimerus elongatus* metacercariae in one bluegill, *Lepomis macrochirus* (standard length = 45 mm) collected in Half Moon Lake, Eau Claire, Wisconsin on 15 October 1983.

Site in fish	Number of metacercariae	Percentage
Caudal fin	85	49
Dorsal fin (soft)	37	21
Dorsal fin (spiny)	8	5
Anal fin	19	11
Pectoral fin (left)	2	1
Pectoral fin (right)	3	2
Pelvic fins	0	0
Muscle (caudal peduncle)	9	5
Muscle (all other)	11	6
Total	174	100

Natural infections in fishes

Bluegills, *Lepomis macrochirus,* and largemouth bass, *Micropterus salmoides,* were the only fishes in Half Moon Lake that harbored *Amphimerus elongatus* metacercariae. Half Moon Lake was not a typical locality to determine the range of host specificity of *A. elongatus* in a truly natural environment, because the fishes of the lake were exterminated with rotenone by the Wisconsin Department of Natural Resources (DNR). Black bullheads, *Ictalurus melas* ($N = 12$), northern pike, *Esox lucius* ($N = 5$), and white crappie, *Pomoxis annularis* ($N = 1$), restocked in the lake by DNR, were not infected. No fish of the families Cyprinidae or Catostomidae were collected in Half Moon Lake despite extensive effort.

Adult bluegills and bass typically were heavily infected with metacercariae. Prevalence in bluegills was 97% ($N = 185$) and bass was 92% ($N = 25$). Intensities of 100–300 or more metacercariae were common. Because of the large numbers of metacercariae, only estimates were made for most fish. However, careful count of cysts in 1 bluegill (45-mm standard length) harboring a typical infection was made to show the actual number and distribution of metacercariae (Table 2).

In July and August, adult bluegill and bass harbored both fully developed metacercariae and newly encysted, incompletely developed meta-

cercariae, indicating that reinfection was possible. Newly hatched bluegill and bass in July had an average prevalence of approximately 20% with typically only 1 or 2 small, incompletely developed metacercariae per infected fish. By October, young-of-the-year bluegill and bass harbored metacercariae considered to be fully developed, although prevalence and intensity remained low.

The effect of temperature on the viability of metacercariae encysted in dead bluegills was determined in order to ascertain whether infections might be acquired by scavengers as well as predatory definitive hosts. Metacercariae did not survive freezing at −10°C for 24 hr. After 48 hr, 100% of metacercariae in dead fish refrigerated at 4°C remained viable, and 50% were able to survive for 12 days at this temperature.

Natural infections in birds

Incidental to this study 5 mallards, *Anas platyrhynchos* Linnaeus, and 4 blue-winged teal, *A. discors* Linnaeus, collected within 50 mi of Half Moon Lake, were examined but were not infected with *A. elongatus.* Because this lake was located in a city park in the center of Eau Claire, Wisconsin, it was not possible to obtain birds for necropsy. However, observations of bird populations were made in order to understand better the ecology of *A. elongatus.* The lake was used by piscivorous summer residents such as herons and kingfishers, and numerous waterfowl considered spring and fall migrants were observed. Furthermore, the city aerated the center of the lake during the winter, and this ice-free area attracted huge flocks of waterfowl, which would ordinarily not use the lake from November through early April.

Geographic distribution of *Amphimerus elongatus*

Although the determination of the geographic distribution of *Amphimerus elongatus* in Wisconsin was beyond the scope of this study, a few bluegills in backwaters of the Red Cedar River, where *Amnicola limosa* also occurred, were infected with metacercariae. Low prevalence and intensity of metacercarial infections precluded an analysis of host specificity in other fishes from the Red Cedar River. Several thousand individuals of the only other hydrobiid snail in the area, *Somatogyrus depressus* (Tryon), were examined for cercariae, but none was infected with *A. elongatus.* Species of fish from numerous localities where hydrobiid snails were absent were not infected.

Experimental infections in fishes

Of the various species of fishes exposed to cercariae in the laboratory, only centrarchid fishes were readily infected with *A. elongatus.* Viable metacercariae were also obtained from several fathead minnows. Only 1 of 20 longnose dace harbored 1 viable metacercaria when examined 24 days postexposure. No other species of fish exposed to cercariae of *A. elongatus* hosted viable metacercariae for more than 7 days. Most insusceptible fish hosts showed no evidence of infection after exposure to over 50 cercariae per fish, but a small percentage of creek chubs, dace, and emerald shiners harbored a few dead, encysted specimens surrounded by a host cellular capsule 1 or more days PE. To illustrate differences in susceptibility, in one experiment 20 specimens each of fathead minnows, juvenile bluegills, and juvenile black bullheads were exposed, in separate containers, to equal numbers of all cercariae shed by 35 *Amnicola limosa.* All bluegills became infected, with a mean intensity of 10.5 metacercariae. Prevalence in fathead minnows was 20% with a mean intensity of 2.3. No black bullheads became infected.

Development of metacercariae was investigated in experimentally infected juvenile bluegills maintained at 22°C. On day 5 PE, cysts measured 123–152 (137) by 62–82 (71) with a parasite cyst wall 7.5 thick. A host-induced cellular capsule was already present around the cyst. The brownish pigmented worms moved actively within the cyst. Eyespots, oral sucker, and empty excretory bladder were clearly visible through the cyst wall. By day 15 PE, excretory concretions were apparent in the bladder, and the brown pigmentation of the body was markedly reduced. On day 20 PE, the ceca were completely developed and filled with cecal platelets (="disk-shaped concretions" of Wallace). The excretory bladder was made conspicuous by black concretions that completely filled the lumen. Cysts measured 180–225 (210) by 100–113 (103). On day 29 PE, metacercariae were identical with fully developed specimens from naturally infected bluegills and bass. Anatomical features, such as the pharynx and oral and ventral suckers, could be observed and measured in encysted specimens. Eyespots were no longer visible. These 29-day-old laboratory-reared metacercariae, when

fed to chicks, developed into adults in liver and pancreas that were morphologically identical to specimens described by Gower (1938a). Viable cysts were found in centrarchids and fathead minnows up to 68 days PE at which time experiments were terminated.

Experimental infections in birds and mammals

Only avian hosts became infected when birds and mammals were fed metacercariae from naturally infected Half Moon Lake bluegills in the laboratory. Mice, rats, and cats were completely refractory to infection. Adult specimens were obtained in chickens and in house sparrows. One-day-old unfed chicks typically attained higher levels of infection than older chickens, but all age groups were successfully infected. Adults were recovered from both liver and pancreas in all hosts harboring more than 1 worm. Adult worms conformed to the description of Gower (1938a), and ontogenetic changes from metacercariae were similar to those described by Wallace (1939).

Discussion

The occurrence of 14 penetration glands in the cercaria of *Amphimerus elongatus* confirms the preliminary description of the cercariae from *Amnicola limosa* in Minnesota (Wallace, 1940). The 3:4:4:3 pattern of ducts formed by the 14 penetration glands indicates that the cercaria of *A. elongatus* is most similar to that of *Clonorchis sinensis* (Komiya and Suzuki, 1964).

The life cycle of *Amphimerus elongatus* is similar to that of most members of the family Opisthorchiidae in which fishes serve as second intermediate hosts and piscivorous birds or mammals are definitive hosts for adults parasitizing the liver and pancreas. Both natural and experimental infections of *A. elongatus* indicated that adults are specific to avian hosts. Thus, it is unlikely that the species represents a public health threat, in spite of its abundance in fishes commonly consumed by humans. Susceptibility of house sparrows to infection was in agreement with the broad host specificity reported by other workers for avian hosts (Gower, 1938a; Boyd and Fry, 1971; Pence and Childs, 1972). Infection of nonpiscivorous birds, like house sparrows and chickens, indicated that natural host range may be limited by avian feeding ecology rather than by host physiology.

Seasonal dynamics in Half Moon Lake were closely associated with the seasonal population cycle of the first intermediate host, *Amnicola limosa.* The large size of the population of *A. limosa,* and the high prevalence of snails infected with *Amphimerus elongatus,* resulted in high cercarial production in the lake. Peak cercarial emergence, however, was confined to a relatively brief but intense period in July, immediately prior to death of the large majority of adult snails. It was interesting that the timing of maximum cercarial emergence meant that most young-of-the-year centrarchids in the lake receive only light infections during their first summer of life. Reinfection of older age class fishes is responsible for most metacercarial infections. These dynamics may be driven by extrinsic factors such as snail ecology and water temperature. However, if juvenile fishes are killed by heavy infections of metacercariae during their first few months of life, this intense selective pressure may be the most important factor responsible for the evolution of the seasonal pattern that exists in Half Moon Lake.

The high prevalence and intensity of *A. elongatus* in Half Moon Lake may be a natural phenomenon, or may be exacerbated by human intervention. Because a portion of the lake was kept ice-free during winter, and birds were fed throughout this period by city residents, the lake supported a much larger waterfowl population for a longer duration than would occur naturally. Greater than normal abundance of metacercariae in fishes may be a result of increased use of the lake by waterfowl, and concomitantly greater amounts of egg-laden feces entering the lake to infect snails. Because the potential pathogenicity of *A. elongatus* adults in birds is of serious concern (Gower, 1938a; Pence and Childs, 1972; Weil et al., 1986), the possible consequences of increased exposure of birds to this parasite due to modification of natural conditions, such as limiting the diversity of fish species in a lake or increasing waterfowl population density, must be considered.

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